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Genetics

The gene for an inherited form of deafness maps to chromosome 5q31

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ABSTRACT **Primary—i.e., nonsyndromal—postlingual deafness is inherited as an autosomal dominant phenotype in a large kindred in Costa Rica. Genetically susceptible individuals begin to lose hearing at low frequencies at about age 10 years, after language and speaking are learned. Deafness inevitably progresses by age 30 years to bilateral hearing loss of all frequencies. Intelligence, fertility, and life expectancy are normal. The family traces its ancestry to an affected founder born in Costa Rica in 1754. We have mapped the gene for deafness in this kindred to chromosome 5q31, between the markers *IL9* and *GRL*, by linkage analysis involving 99 informative relatives.**

Human deafness is a major medical and public health concern. A generation ago, most congenital deafness was attributable to viral diseases during pregnancy. Now that maternal viral diseases are far less common, perhaps 50% of deafness in newborns has a genetic basis (1, 2). Probably an even higher proportion of deafness among older children and young adults is genetically influenced. However, the genetics of human deafness is complex and heterogeneous. There are at least 32 genetic forms of primary human deafness (i.e., deafness not secondary to some other disease), some of which may themselves be heterogeneous (3). In addition to genetic heterogeneity across affected families, deafness among affected children in the same family may be due to different genes, because deaf persons often marry each other. Furthermore, some congenital forms of deafness are associated with severe abnormalities, sometimes leading to early death and often to limited family size, so for these conditions few families with more than one affected child are to be found. Finally, many genetic forms of deafness are variable in expression with incomplete penetrance. It is probably not surprising, therefore, that heretofore no genes have been mapped for primary human deafness.

Inherited low-frequency hearing loss (*LFHL1*; no. 124900 in ref. 3) is an autosomal dominant, fully penetrant, sensorineural deafness originally described in an extended Costa Rican kindred (4). In this kindred, low-tone deafness begins at about age 10 and progresses by age 30 in males and females to profound, irreversible, bilateral deafness involving all frequencies. Impedance tests suggest normal stapedial reflexes and no mechanical damage (5). Tone decay and other audiometric studies indicate normal retrocochlear function. Speech development before onset of deafness is normal, as are intelligence, fertility, and life expectancy.

Deafness in the Costa Rican kindred has been traced back eight generations to Felix Monge, who was born in Costa Rica in 1754. Testaments by Felix Monge and two of his brothers indicate they were deaf but were born hearing. Felix Monge's sibship was the seventh generation of their family in Costa Rica. Their ancestors migrated to Costa Rica from Jerez de la Frontera, Spain, about 1600. Most of the descendants of Felix Monge still live near Cartago, Costa Rica. At least 150 living adults from the family are informative for linkage analysis. For this study, relatives were considered informative if they either were diagnosed as deaf or were older than age 25 years with no symptoms of hearing loss. Ninety-nine of these informative relatives are included in this report.

MATERIALS AND METHODS

Clinical evaluation of deafness was carried out by audiometric testing at the Centeno Guell Auditory Testing Facility in San Jose, Costa Rica, as described (4, 5). Audiologic testing was conducted in an Industrial Acoustics Corporation sound chamber (model 400-SER). Interior noise levels did not exceed 40 decibels, scale A. A clinical audiometer (Maico, model MA-22) and a portable audiometer (Maico, model MA-16) were used for pure tone and bone conduction testing at 250 Hz through 8000 Hz. An impedance bridge (Teledyne, model TA-3D) was employed for all tympanometry and acoustic reflex testing. All audiometric equipment was calibrated before each period of testing. Before audiometric evaluation, subjects were given otoscopic examinations to determine the condition of their ear canals and then given the opportunity to practice the required tasks. Pure tone and bone conduction thresholds were measured using the modified Hughson–Westlake technique (6). Frequencies tested by air conduction were 250, 500, 100, 2000, 3000, 4000, 6000, and 8000 Hz. Frequencies tested by bone conduction were 250, 500, 1000, 2000, 3000, and 4000 Hz.

Affected relatives older than age 25 in the kindred had pure tone air conduction hearing threshold levels above 50 decibels for all frequencies. Affected relatives younger than age 20 had mild to moderate hearing losses through 1000 Hz but retained normal or near-normal thresholds for higher frequencies. By age 30, thresholds were at severe levels across the entire frequency range, leading to flat profound hearing loss by age 40. Audiometric configurations for right and left ears were similar, with all deaf persons affected bilaterally. A minimal criterion for deafness in this kindred was a hearing threshold greater than 50 decibels at 250 Hz and 500 Hz. Relatives younger than age 25 with no apparent symptoms were not included in the analysis.

Whole blood for 99 informative relatives was drawn into acid citrate dextrose, lymphoblastoid cell lines were prepared, and DNA was extracted using methods previously described (7, 8). Southern hybridizations were carried out according to standard procedures (9–11). Amplification and electrophoresis of sequences containing microsatellite polymorphisms were carried out by methods described for these loci (12–15). In Costa Rica, where ^{32}P was not available, sequences containing microsatellite polymorphisms were amplified without labeled nucleotides, then electrophoresed

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Abbreviations: lod, logarithm of odds; cM, centimorgan(s).

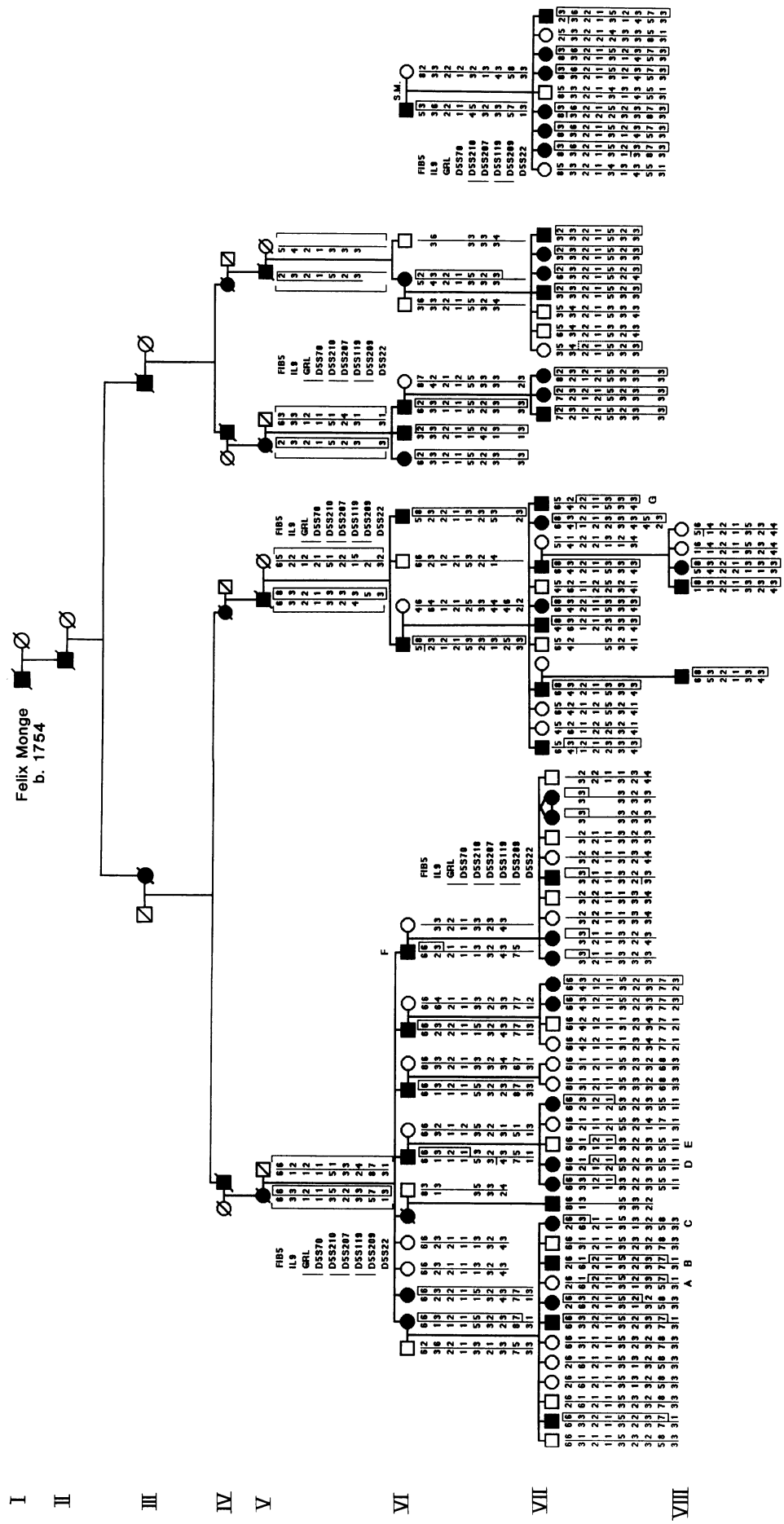

FIG. 1. Linkage of deafness in the Monge kindred to markers on chromosome 5q31. Dark symbols indicate deaf persons; symbols with diagonal slashes represent deceased persons. The position of the "S.M." branch in the kindred is not certain. Genotypes of some deceased persons are suggested on the pedigree in brackets, but these inferred genotypes were not included in the statistical analysis. Boxes indicate the haplotypes apparently linked to deafness in each branch of the kindred. By multipoint analysis, odds in favor of linkage of deafness to the region between *IL9* and *D5S210/D5S207* are $>10^{12}$:1. Recombination events in persons A, C, E, and F indicate that the deafness gene lies above *GRL*; recombination events in persons B, D, and G indicate that the deafness gene lies below *IL9*. The distance between GRL and IL9 is ≈7 centimorgans (cM).

Table 1. Polymorphisms on chromosome 5q linked to deafness in the Monge kindred

Locus	Allele	θ	Primer or probe/enzyme
FIB5	8		AAG GTG TTC TTT GCA TGT TCA CC GTA ATG TGT TCT ATC TAG TTC AAC G
		0.12	
IL9 (SE9)	6		AGG TCC AGG CTA GCT CAT GT CTA ATG CAG AGA TTT AGG GC
		0.07	
GRL	2		OB7/*Bcl* I
		0.00	
D5S70	2		TP5E/*Taq* I
		0.07	
D5S210 (Mfd122)	5		ATG CAG AAT CTA CAA GGA CC CTT TAA CAT CCT TTA ACA GC
		0.00	
D5S207 (Mfd42)	3		TTG GAA GCC TTA GGA AGT GC AAG AAT TCT AGT TTC AAT ACC G
		0.14	
D5S119 (Mfd6)	4		TCC TAC CTT AAT TTC TGC CT GCA GGT TGT TTA ATT TCG GC
		0.00	
D5S209 (Mfd116)	8		CTG CAG TAG AAA GGC AGA GT TGC AGC ACC AAA CAC CAA GT
		0.11	
D5S22	3		JO205H-C/*Msp* I

θ, Approximate distance between adjacent markers.

in 18–20% acrylamide gels, and stained with silver nitrate or ethidium bromide.

Autosomal dominant transmission of deafness in the Monge kindred was confirmed previously by segregation analysis (4). Linkage was evaluated using LIPED (16), postulating a rare autosomal dominant allele (q = 0.01) with complete penetrance and no sporadic cases. Multipoint analysis was carried out using LINKAGE (17). Only genotypes that were obtained explicitly for each person were included in the linkage analysis: no inferred genotypes nor any haplotypes were included in the statistical analysis.

RESULTS

Primer sequences or probe/enzyme combinations for markers on chromosome 5q are listed in Table 1 (see refs. 12–15 for allele frequencies). Order of markers and approximate distances between adjacent markers are based on data from CEPH (ref. 13; unpublished data) and from the Monge kindred. Relative orders of *D5S210* vs. *D5S207*, of *D5S119* vs. *D5S209*, and of *GRL* vs. *D5S70* cannot be determined from our data so far.

Coinheritance of deafness with chromosome 5q markers in the Monge kindred is shown in Fig. 1. Haplotypes and genotypes for deceased persons are indicated in Fig. 1 but were not included in the statistical analysis. Two-point logarithm of odds (lod) scores for linkage of deafness to markers in this region are indicated in Table 2. Multipoint analysis of the 14-cM interval defined by *IL9* and *D5S210/D5S207* strongly suggests that the *LFHL1* gene is between these markers. The maximum lod score for a gene proximal to *IL9* is 9.95; the maximum lod score for a gene within the interval is 12.42; the maximum lod score for a gene distal to *D5S207/D5S210* is 7.46. The maximum lod score within the interval occurs ≈5 cM distal to *IL9*, which corresponds to a locale slightly proximal to *GRL*.

Table 2. Maximum two-point lod scores (Z), maximum likelihood recombination fractions (θ), and 95% confidence intervals for θ for linkage of deafness to chromosome 5 markers

Marker	Z	θ	95% confidence interval
FIB5	6.63	0.06	(0.01, 0.12)
IL9	13.55	0.06	(0.01, 0.12)
GRL	2.65	0.10	(0.02, 0.30)
D5D210/D5S207	7.50	0.13	(0.05, 0.31)

Seven persons with informative recombination events place the deafness gene *LFHL1* between *IL9* and *GRL*. In Fig. 1, persons A, C, E, and F indicate that *LFHL1* is proximal to *GRL*; B, D, and G indicate that *LFHL1* is distal to *IL9*.

DISCUSSION

Deafness in this kindred appears to be determined by a gene between *IL9* and *GRL*, an interval of ≈7 cM. Because *LFHL1* is primary deafness, with no associated abnormalities, the normal function of the *LFHL1* gene may be specific to hearing. Other occurrences of sensorineural deafness, whether inherited or apparently sporadic, may be due to other mutations at the *LFHL1* locus (3, 18). Linkage information from the Monge kindred will permit this hypothesis to be tested in other families. The *LFHL1* gene appears not to be identical to the gene for Treacher Collins syndrome, a complex mandibulofacial disorder sometimes involving deafness, which maps distal to *GRL* and, hence, distal to the *LFHL1* gene (19, 20).

Physical maps of human chromosome 5q indicate that *IL9* and *GRL* map to 5q31 (21, 22). Genes coding for early growth response (*EGR1*), antigen CD14, and endothelial growth factor (*FGFA*) are located between *IL9* and *GRL* (21). This region of human chromosome 5q is homologous to a portion of mouse chromosome 11. The mouse shaker-2 locus maps to this region, but outside the interval defined by Sparc and the interleukin loci (23). If genes in this region are in the same order in mouse and humans, the shaker-2 locus would be outside the critical region of linkage for *LFHL1* (21).

The *LFHL1* gene can be further localized by mapping deafness in the Monge kindred relative to additional markers in this region of chromosome 5q. Given that ≈150 informative relatives have agreed to participate (of whom 99 have been sampled thus far), this large kindred offers the potential of mapping this gene to a resolution of ≈1 cM. Coding sequences in close physical proximity to markers showing no recombination with *LFHL1* can then be investigated.

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